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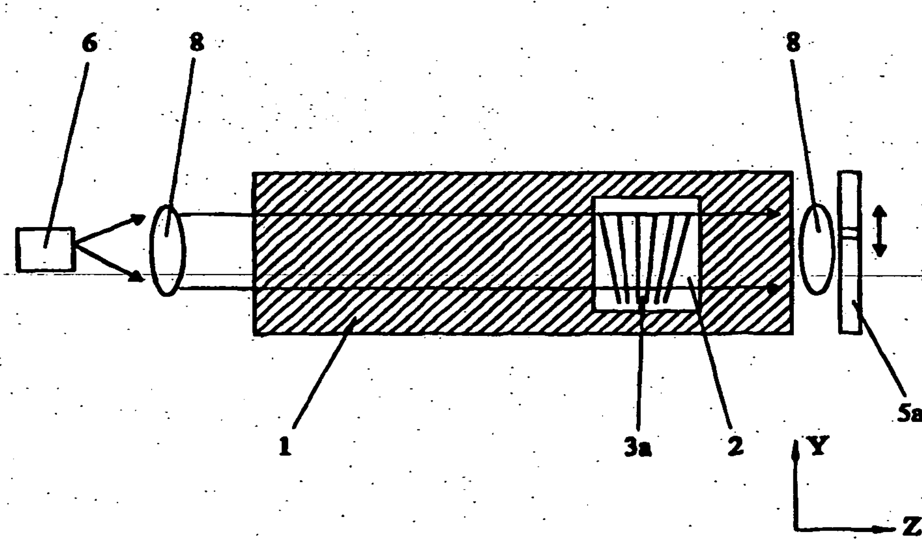

(57) Abstract

A sensor system for use in surface plasmon sensing which is capable of coupling a single propagating optical waveguide mode t a surface plasmon polariton mode mediated by an integrated optical grating positioned between the modes. The system provides optical coupling at a single wavelength and angle whilst simultaneously providing a wide range of excitation conditions to be monitored in real time.

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COUPLED MODE OPTICAL SENSOR

The present invention relates to a surface plasmon sensor, in particular to an optical waveguide sensor for the detection of materials that preferentially bind to a metal surface.

Detection of molecules that selectively bind physically or chemically to a metal surface has been demonstrated through the attenuated total reflection (ATR) method using surface plasmon polaritons (SPP) (see Chegel *et al*: Sensors & Actuators, B48, (1998) 456 and Sakai *et al*: Sensors & Actuators, B49, (1998) 5). The suggestion that SPP may be used for biological assays using surface bound antigens (Flanagan & Pantell: Electronics Letters, 20, (1984) 968) has led to the commercialisation of laboratory instruments for biological and other assays using ATR.

SPPs are surface bound electromagnetic waves having optical propagation qualities that are extremely sensitive to the dielectric conditions within a distance of a few nanometres from a metal surface. In ATR configurations, the intensity of the light reflected from the surface of the metal is attenuated when a critical angle of incidence is obtained. The critical angle at which coupling to the SPP occurs can be changed if an analyte of interest is selectively bound to the metal surface. In biosensing applications, it is of particular interest to prepare the metal surface with a monolayer of a suitable receptor molecule for the analyte of interest. In this form, the system has a particular SPP propagation constant (β_{SPP}) which corresponds (at a particular wavelength) to a critical angle for attenuated reflection. On binding of a monolayer of the analyte, the critical angle changes may be measured. The extreme sensitivity to the near surface region has made the

ATR method very attractive for specific sensing of monomolecular layers.

It has been hitherto necessary to use transmission of light through a prism or to use direct incidence onto a grating in order to excite (or couple to) the SPP. A number of variations using the fundamental physical principles of the prism coupling technique have been applied to biosensing applications. One of two methods is generally employed. In the first method, a single wavelength beam is brought to incidence on the metal/dielectric boundary and the incident angles at which reflection is attenuated are used to detect the SPP (Melendez *et al*: Sensors & Actuators, B35 to 36, (1996) 212). The single wavelength beam may be a converging or diverging source to provide a spread of angles within the incident beam (US-A-4844613). In the second method, a broad spectral source is used at a single angle or a fixed range of angles and a spectral measurement of attenuated reflection is used to detect the SPP (see Jorgensen and Yee: Sensors & Actuators, B12, (1993) 213 and Cahill *et al*: Sensors & Actuators, B45, (1997) 161).

Although well understood, the traditional prism method is regarded as somewhat bulky and inflexible and has not successfully penetrated to the mass market. More recent developments have aimed to simplify this technique and wherever possible to integrate the components of the system. For example, a commercial fibre optic SPR probe has been developed which is based on the multi-wavelength spectral method of detection (Jorgensen & Yee: Sensors & Actuators, B12, (1993) 213). An integrated system combining the single wavelength source (providing a fixed range of angles) and a diode array detector has also been developed (Melendez *et al*: Sensors & Actuators, B35-36, (1996) 212). A further development of the method uses a metal-clad substrate mode

waveguide and focussing of the beam into the waveguide to achieve a spread of coupling conditions for the beam within the spread of incidence angles provided at the waveguide/metal interface (US-A-5485277).

In each of these known techniques, the light incident on the metal/dielectric interface comprises either a plane wave beam of light or comprises light whose ray components impinge on the metal/dielectric boundary at a range of angles. It is critical that alignment of the incident beam is maintained between sample changes.

A planar waveguide format has also been proposed in relation to surface plasmon sensors. Excitation of SPPs via distributed coupling between the SPP and a planar dielectric waveguide mode is one such possibility. The basic structure comprises a dielectric waveguide onto which is deposited a metal film in contact with a superstrate medium which could contain analyte. Theoretical modelling for such a structure (see Harris and Wilkinson, Sensors and Actuators, B29, (1995), 261) shows that a resonance condition can be achieved only for particular values of the superstrate refractive index. Under this condition, the optical throughput of the dielectric waveguide is considerably attenuated and this forms the basis of a sensing function. The method requires very precise fabrication and suitable devices can only be fabricated from a severely limited range of materials (a waveguide top cladding material of very low refractive index is required for example) and so the method has not been taken up widely. In addition, the dynamic range (the range over which the device can be used) is very narrow. An optical fibre version of the device is also known (see Homola, Sensors and Actuators, B29, (1995), 401).

There is generally a need for a method based on measurement of SPP which is an improvement over the ATR method and the planar waveguide method of the prior art. The present invention seeks to address this need by providing optical coupling at a single angle and single wavelength using for example a suitable low cost laser diode whilst simultaneously providing a wide range of possible excitation conditions to be monitored in real time. Further advantages of the invention will become apparent from the description hereinafter.

Thus viewed from one aspect the present invention provides a sensor system for use in surface plasmon sensing comprising:

an optical waveguide structure capable of providing a single optical mode of transverse magnetic (TM) polarisation;

a surface plasmon polariton (SPP) generating surface adapted so that its dielectric properties are modified in the presence of an analyte; and

an integrated optical grating.

Typically a sensor system of the invention may be formed by vertical layer integration on a common substrate to provide a device for chemical, biochemical or biological sensing. In use, the sensor system couples the single propagating optical waveguide mode to a surface plasmon polariton mode mediated by the integrated optical grating positioned between the modes. For this purpose, the optical waveguide structure preferably provides no more than a single optical mode of TM polarisation.

One of the advantages of the invention is that in use there is neither a requirement to monitor or control the angle of incident radiation nor a requirement to provide a broadband source of radiation. The sensor system of the

invention provides simultaneous alignment of the propagation of the source radiation and the direction of propagation of the SPP, whilst dispensing with any requirement for multiple wavelength sources or resolving spectrometers or bulk optical components (*eg* prisms and beam transporting components). The invention may take the form of a disposable or recyclable sensor system made from a wide selection of materials and is operable without moving parts and suitable for specific detection of biological material during for example immunoassay.

A further advantage of the invention is that coupling of radiation to the sensor system is relatively straightforward and tolerant to relative misalignments of source and waveguide structure. End-fire coupling using a polarised source of electromagnetic radiation (*eg* a laser diode source) is the most convenient method of irradiation.

The optical waveguide structure is preferably a slab or channel optical waveguide structure. It may be fabricated from a suitable combination of optically transmissive materials. Preferably the materials are fabricated in layers and the number, thickness and refractive index of the layers determines the characteristics of the optical waveguide structure as a whole. Preferably the materials may be deposited in a laminar fashion with known thicknesses and well characterised refractive indices. Silicon oxynitride (SiON) is a particularly preferred material. The fabrication of a suitable waveguide structure may be carried out using techniques well-known to those skilled in the art.

Preferably each layer is of a thickness in the range 0.5 to 5μm, particularly preferably 0.5 to 1μm. The refractive index of each layer is preferably in the range 1.4 to 1.65.

The grating structure preferably provides a range of coupling conditions. Preferably it takes the form of a surface relief grating structure patterned onto the surface of the sensor system. Particularly preferably, the grating structure is a fanned grating structure. The principles of grating coupling between vertically integrated dielectric waveguides have been demonstrated (see, for example, Alferness *et al*: Applied Phys. Lett., 55, (1989) 2011 and Butler *et al*: J. Lightwave Tech., 16, (1998) 1038) and methods for preparing grating structures (*eg* etching, holography) are well-known.

In one embodiment of the invention, the guided mode (GM) is excited with a guided slab waveguide mode of fixed fanning angle, θ. The diverging beam crosses beneath the grating and provides a continuous spectrum of possible coupling conditions from the beam centre to either side. Parts of the propagating waveguide mode whose propagation constant matches that of the effective grating spacing required for phase matching with the SPP will be attenuated. Mirror images of the attenuated light will be observed on either side of the propagation axis. A diode array detector may be used to provide an output signal for measurement. Upon detection of the analyte, the dark bands will move laterally across the array by an amount determined by the changing dielectric qualities of the surface region.

Advantageously, a combination of fanned grating and fanned beam may be used in the same embodiment. At larger fanning angles (α), the simultaneous beam fanning will compensate for possible loss of coupling efficiency between the GM and the SPP.

In a further embodiment, a fanned grating structure may be used in reflection mode using an expanded beam incident

through a sufficiently transparent analyte medium. The reflected image will contain a dark band corresponding to the lateral position at which the SPP wavevector matches the incident beam wavevector component in the longitudinal direction defined as the direction in the plane of the grating normal to the lines of the grating.

A particular advantage of this embodiment is that the fanning angles (α) of the grating may be chosen to suit any particular application thereby providing a device of greater dynamic range than is possible with conventional devices. The range of grating spacings provided by the fanning and the width of the grating will in general determine the dynamic range of the device and allow a wide range of refractive indices of analyte medium to be advantageously used with a single design.

The characteristics of the SPP generating surface may be modified in the presence of an analyte. This may be caused by chemical, biochemical, biological or physical interaction with the analyte. In use, the SPP generating surface may be provided with a monolayer of a binding partner (*eg* receptor) to the analyte of interest. Examples of such interactions are well-known to those skilled in the art and include antigen-antibody interactions which may be used as the basis of an immunoassay.

In one embodiment, the SPP generating surface may comprise a thin metal film deposited onto the surface of the sensor system so that the upper metal surface may contact an analyte medium such as for example an aqueous medium or blood. Typically therefore the device is adapted to sense changes in the effective refractive index of the dielectric superstrate close to the value of water (1.33).

Advantageously the metal film is deposited over the surface relief grating. Preferably the metal film may be gold, silver or aluminium, particularly preferably it is gold. Methods for depositing thin metal films on the sensor substrate are well known to the skilled person.

The fundamental condition required for coupling between the GM and the SPP at a given wavelength (λ), is given by the following phase matching condition

$$\left|\beta_{spp} - \beta_{gm}\right| = K \qquad (1)$$

where, β_{spp} and β_{gm} are the propagation constants of the SPP and the GM respectively and K is the grating wavenumber $(K = 2\pi/\Lambda)$. When this equation is satisfied, optical power may transfer between the two modes. If, preferentially, the power originates in the GM and transfers to the SPP, it will be strongly absorbed. Whilst the coupling allows in principle for periodic transfer of light between the two modes along the propagation direction there will be a greatly reduced reverse transfer of light from the SPP to the GM within one coupling length.

For a given difference in propagation constants between the GM and the SPP a characteristic grating period (Λ), will satisfy this condition. In the preferred fanned grating embodiment or in the alternative fanned beam embodiment, the coupling conditions will therefore be satisfied at a particular point across the grating in the y direction (see for example Figure 2).

The presence of a bound analyte within a few nanometres of the metal surface can be modelled as a change to the

effective refractive index ($\sqrt{\varepsilon_{eff}}$, where ε_{eff} is the effective relative permittivity) of the semi-infinite medium above the surface. In this case, the propagation constant of the SPP varies according to the following:

$$\beta_{spp} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}}$$

(where ω and c are the frequency of the incident light and the free-space speed of light respectively and ε_m is the relative permittivity of the metal).

In an embodiment of the invention, the waveguide structure may be fabricated such that the value of β_g is within a range close to the value of β_{SPP}. This is achieved by controlling the thickness and refractive index of the layers of the waveguide structure.

In a further embodiment of the invention, β_g is slightly greater than β_{SPP} for the SPP without an analyte bound layer. A grating period is provided for this condition so as to satisfy phase matching according to equation (1). On binding of the analyte, β_{SPP} is raised by a specific amount determined primarily by the refractive index and thickness of the analyte layer. Over a range of degrees of binding of the analyte, the phase matching condition is achieved over a range of lateral positions of the grating. Where this condition is reached, radiation is coupled from the GM into the SPP via the evanescent fields extending through the structure and radiation is absorbed from the GM into the metal film. In addition, radiation may also be scattered into radiation modes above the metal surface. Thus in one embodiment, coupling may be confirmed by simultaneous measurement of the reduction in propagating intensity of the

GM using a first detector and the appearance of scattered light above the sensor metal surface using a second detector.

In a preferred embodiment of the device of the invention an additional waveguide structure (*eg* slab or channel waveguide structure) is fabricated below or adjacent to the optical waveguide structure described hereinbefore and is capable of functioning as a reference waveguide. This additional waveguide structure may be used to provide continuous measurement of the output intensity of the structure.

In a further embodiment of the invention, a plurality of sensor components are integrated on a single substrate. Each sensor component may be addressed individually from a separate optical source or all sensor components may simultaneously be addressed from a common optical source.

Viewed from a further aspect the present invention provides a method for determining the presence or amount of an analyte comprising:

positioning a sensor system as hereinbefore described adjacent to a radiation source and a first detector;

exposing the SSP generating surface to the analyte; and

monitoring the output radiation from said waveguide structure.

Typically, the first detection means will be a plurality of diodes (*eg* in an array). The characteristics of the output radiation will depend on the phase matching of the propagation constants β_g and β_{SPP}. As explained above, phase matching of β_g and β_{SPP} depends on the characteristics of the bound analyte layer. The method may be used therefore to give real time measurements of analyte binding since the diode array may be scanned continuously and data accumulated.

In a further embodiment, a plurality of different sensing materials (*eg* different antibody systems) may be applied to different regions of the metal surface and the emission of light from the metal surface of a sensor may provide further utility. With knowledge of the region in which each different test system (antibody) has been applied, the position of the emission light will indicate the particular sensing system which has responded to the test material. If the detector comprises a two-dimensional array of detector elements (analogous to pixels), the sensing system or systems which are responding may be determined from the position at which light emission occurs. In this manner a number of tests may be carried out simultaneously.

In a preferred embodiment, the sensor substrate may be housed in a device similar to that described in WO-A-99/63330 (Farfield Sensors Limited).

The invention will now be described in a non-limitative sense with reference to the accompanying Figures:

Figure 1 is a side view of one embodiment of the sensor chip substrate of the invention;

Figure 2 is a plan view of a sensor substrate of one embodiment of the invention;

Figure 3 illustrates the variation of the grating pitch (Λ), as a function of distance (y) with a fan angle (α) of the grating;

Figure 4 illustrates in plan view the means to provide a fanned beam across a grating of fixed period;

Figure 5 illustrates a plurality of sensor components integrated on a single substrate;

Figure 6 illustrates a typical design of grating spacing Λ (ordinate in units of μm) required for phase

matching as a function of superstrate effective index $\sqrt{\varepsilon_{eff}}$ (abscissa).

As illustrated in Figure 1, a slab waveguide structure comprising three dielectric optical layers in a vertical dielectric stack is indicated generally by reference numeral 1. An optical source of polarised radiation is indicated by reference numeral 6 and an optical transforming component by 8 (*eg* a lens). The single optical mode of the structure propagates as a slab waveguide mode of transverse magnetic (TM) character. The transverse optical field distribution of the waveguide mode is shown schematically as reference numeral 7. It has an evanescent component which extends through the thickness of the sensor system. A surface relief grating 3 is patterned onto the surface of the sensor system.

The SPP generating surface comprises a thin metal film (*eg* gold) 2 deposited onto the surface of the sensor system over the surface relief grating 3 so that the upper metal surface makes contact with the analyte medium (such as, for example, a water based medium or blood). The deposited metal film coats the grating and adopts its spatial periodicity (Λ) in the longitudinal direction.

When the degree of binding of an analyte is such that the phase matching condition (see equation (1)above) is achieved, radiation is coupled from the GM into the SPP via the evanescent fields extending through the structure and radiation is absorbed from the guided mode and into the metal film. In addition, radiation may also be scattered into radiation modes above the metal surface. Thus the detection of coupling is confirmed by simultaneously measuring the reduction in propagating intensity of the GM using detector 5

and an appearance of scattered light above the sensor metal surface is measured by detector 4 (*eg* an optical fibre).

With reference to Figure 2, fanned relief grating 3a is patterned to give a continuous variation in grating period ($\Lambda(y)$ see Figure 3) across a laterally expanded beam from source 8 propagating in the underlying single slab waveguide structure. The fanned grating structure 3a smoothly decreases in grating wavevector K as a function of lateral direction y. Propagation of the expanded guide beam is along z. The output image of the guided mode (near field or far field) is imaged with a linear, diode detector array 5a. Across the array, phase matching of the guided mode to the SPP mode is indicated with a band of relatively low intensity spanning a few pixels to either side of the central position. This band moves when detecting analyte. Its relative position may be correlated with the relative difference in propagation constant β_g and β_{SPP} between the guided mode and the SPP mode respectively during sensing. Thus the device is capable of constantly monitoring this difference as a function of time. The fabrication of the grating structure and design of the SPP mode effective index can be closely controlled. Thus the initial position of the dark band (when a known cladding material is used *eg* air or other medium) may be used as a measure of the absolute effective index of the guided mode. This simultaneously provides a check on the quality of fabrication over a batch of sensors.

Figure 4 illustrates schematically an embodiment in which a fanned beam is used across an optical grating of fixed period 3. The linear detector array 5a is used to detect phase matching of the guided mode to the SPP mode in the form of dark bands 40 which moves when detecting analyte.

Figure 5 illustrates a preferred embodiment of the assembly of the invention in which three sensor systems are integrated onto a common single substrate.

EXAMPLE

A sensor system of the invention may be fabricated onto silicon wafers. In one suitable design, the following layer properties will provide a single slab waveguide TM mode at 780 nanometres. Onto a silicon wafer is deposited a silicon oxynitride of refractive index 1.47. The thickness should not be less than 2 micrometres. A second silicon oxynitride layer is of refractive index 1.50 and thickness 1 micrometre. A third layer is a silicon oxynitride layer of refractive index 1.495 and thickness 1 micrometre. Into this surface cladding layer, a surface relief grating may be patterned by reactive ion etching after suitable photolithography. Onto this structure, a gold layer is deposited to a typical thickness of 50nm.

The fanning angles of the grating (α) can be chosen to suit a particular application. The range of grating spacings provided by the fanning and the width of the grating will determine the dynamic range of the device. Thus, a wide range of refractive indices of analyte medium can be used with a single design. The design below assumes a wavelength of 650 nm, a guided mode effective refractive index of 1.55 and a gold metal layer and supposes that a range of effective indices between 1.33 and 1.38 are required to be spanned by the grating. Figure 6 illustrates the grating spacing (μm) required for phase matching as a function of superstrate refractive index.

The fanning angle (α), required to accommodate such changes over a lateral distance (y), of 4 mm for example, would be 0.115 degrees.

CLAIMS

1. A sensor system for use in surface plasmon sensing comprising:

an optical waveguide structure capable of providing a single optical mode of transverse magnetic (TM) polarisation;

a surface plasmon polariton (SPP) generating surface adapted so that its dielectric properties are modified in the presence of an analyte; and

an integrated optical grating.

2. A sensor system as claimed in claim 1 formed by vertical layer integration on a common substrate.

3. A sensor system as claimed in either claim 1 or claim 2 wherein the optical waveguide structure is capable of providing no more than a single optical mode of TM polarisation.

4. A sensor system as claimed in any preceding claim wherein the optical waveguide structure is a slab or channel waveguide structure.

5. A sensor system as claimed in any preceding claim wherein the optical waveguide structure is deposited in a laminar fashion.

6. A sensor system as claimed in any preceding claim wherein the optical grating is a surface relief grating structure patterned onto the surface of the optical waveguide structure.

7. A sensor system as claimed in claim 6 wherein the surface relief grating structure is a fanned optical grating.

8. A sensor system as claimed in claim 7 wherein the fanned optical grating is patterned to give a continuous variation in grating period across a laterally expanded beam.

9. A sensor system as claimed in any preceding claim further comprising a single wavelength and fixed single angle beam source.

10. A sensor system as claimed in any preceding claim further comprising means for providing a diverging beam of a fixed fanning angle for exciting the guided mode of the waveguide structure.

11. A sensor system as claimed in claim 10 wherein the analyte comprises a chemical, biochemical, physical or biological stimulus.

12. A sensor system as claimed in either of claims 10 or 11 wherein the SPP generating surface is provided with a monolayer of a binding partner to the analyte.

13. A sensor system as claimed in any preceding claim wherein the SPP generating surface is deposited over the integrated optical grating.

14. A sensor system as claimed in any preceding claim wherein the SPP generating surface is a thin metal surface.

15. A sensor system as claimed in claim 14 wherein the thin metal surface is gold, silver or aluminium, preferably gold.

16. A sensor system as claimed in any preceding claim wherein the waveguide structure is fabricated such that the propagation constants of the SPP and of the guided mode are nearly or exactly the same.

17. A sensor system as claimed in any preceding claim further comprising a reference waveguide structure.

18. A sensor system as claimed in any preceding claim which is adapted to sense changes in the effective refractive index of the SPP generating surface.

19. A sensor assembly comprising a plurality of sensor systems as defined in any preceding claim intergrated onto a common substrate.

20. A method for detecting changes in a localised environment caused by the introduction of or changes in an analyte, said method comprising:

positioning a sensor system or assembly as defined in any of claims 1 to 19 adjacent to a radiation source and one or more detectors;

exciting the guided mode of the waveguide structure with a beam using the radiation source;

monitoring a characteristic of the output radiation using the one or more detectors;

exposing the SSP generating surface to the analyte in the localised environment;

monitoring changes in the characteristic of the output radiation from said waveguide structure; and

relating changes in the characteristic of the output radiation from said waveguide structure to changes in the localised environment.

21. A method as claimed in claim 20 wherein the characteristic of the output radiation is its intensity.

22. A method as claimed in either of claims 20 or 21 wherein the guided mode is excited using a diverging beam having a fixed fanning angle.

23. A method as claimed in any of claims 20 to 22 further comprising the step of correlating changes in the propagation constants of the SPP and of the guided mode to the changes in the characteristic of the output radiation from said waveguide structure.

24. A method as claimed in any of claims 20 to 22 comprising the step of correlating changes in the effective refractive index of the SPP generating surface to the changes in the characteristic of the output radiation from said waveguide structure

25. A method as claimed in any of claims 20 to 24 in which the optical grating is a fanned optical grating.

26. A method as claimed in any of claims 20 to 25 wherein coupling of the SPP and of the guided mode is confirmed by simultaneous measurement of (1) the reduction in propagating intensity of the guided mode using a first detector and (2) the appearance of scattered light above the SPP generating surface using a second detector.

27. Use of a sensor system as defined in any of claims 1 to 18 for chemical, physical, biological or biochemical sensing.

28. Use of a sensor system as claimed in claims 27 as an immunoassay.

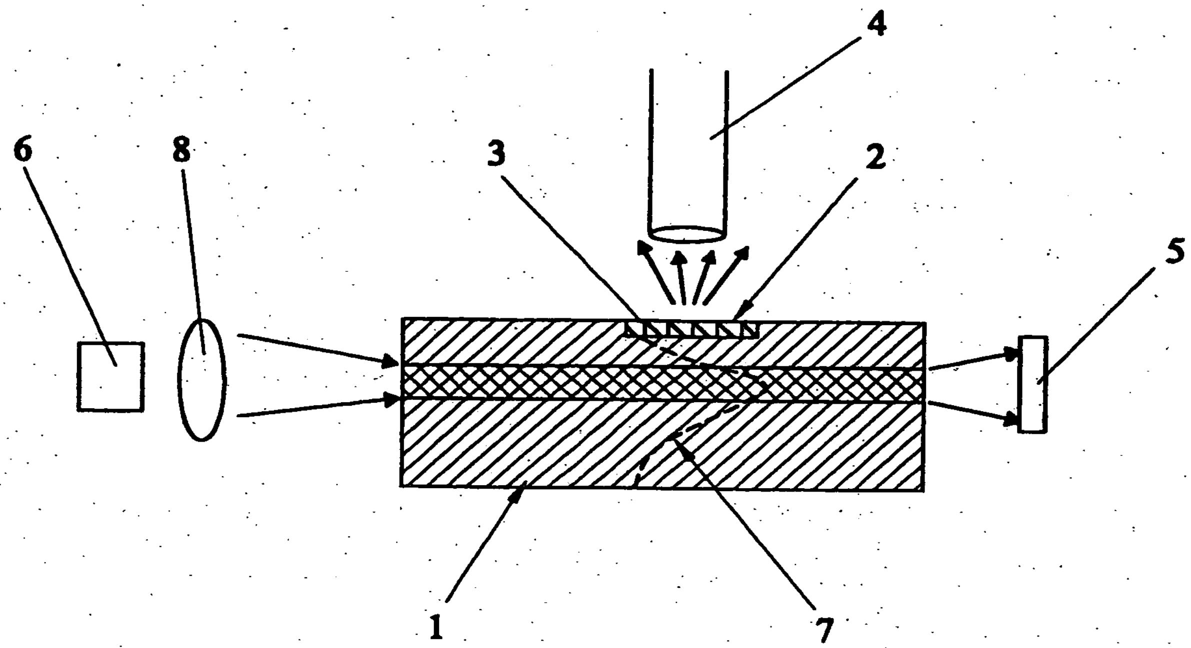

FIG. 1

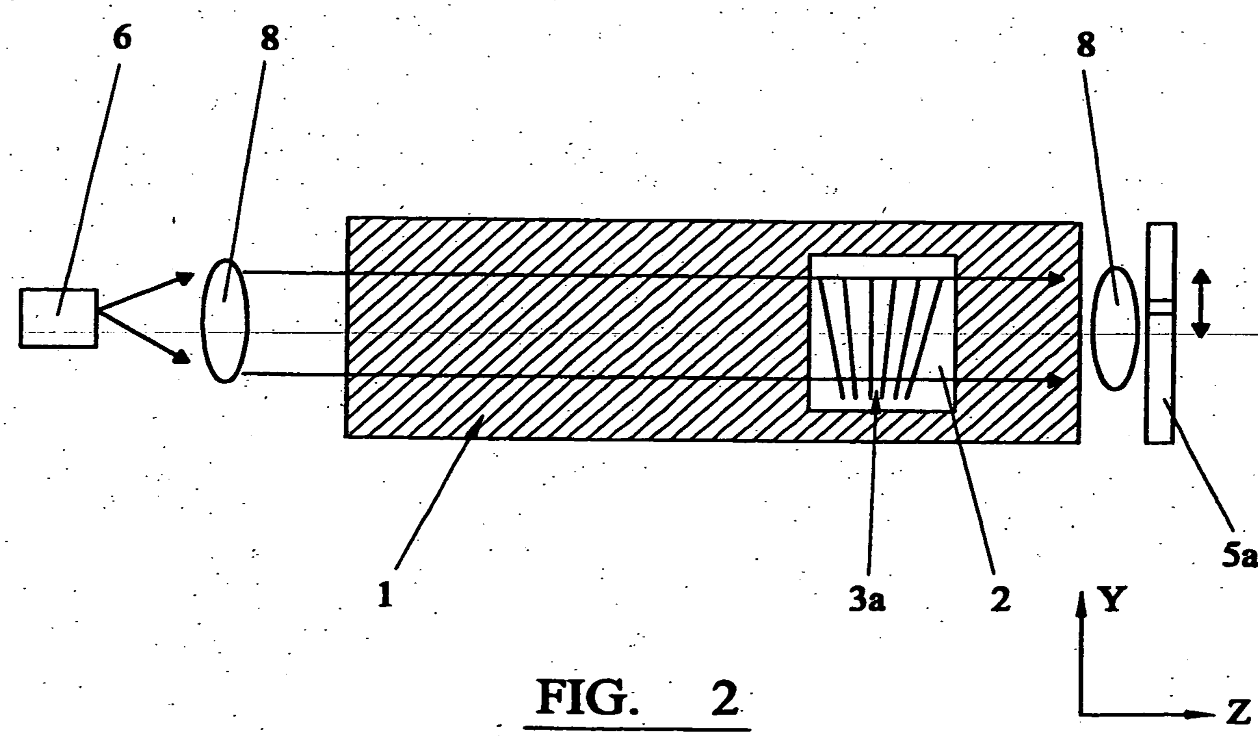

FIG. 2

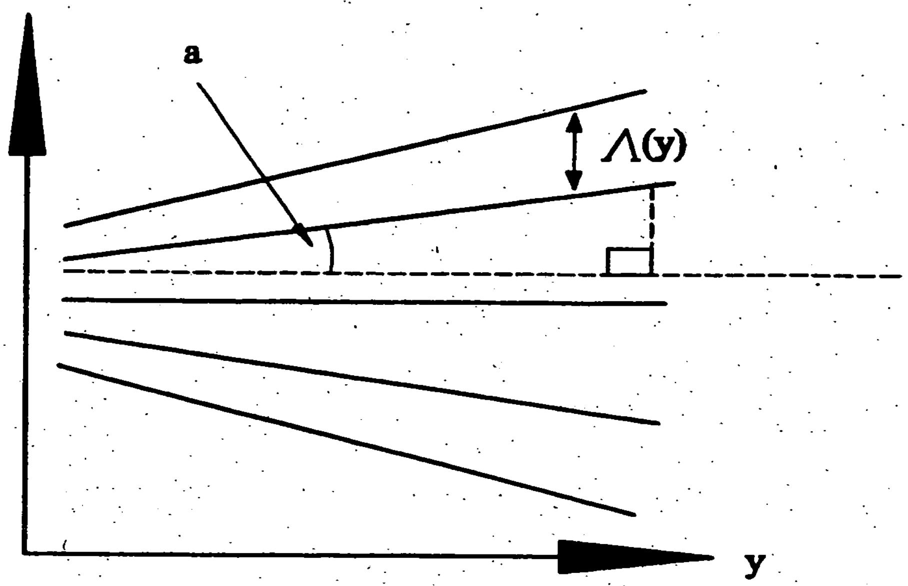

FIG. 3

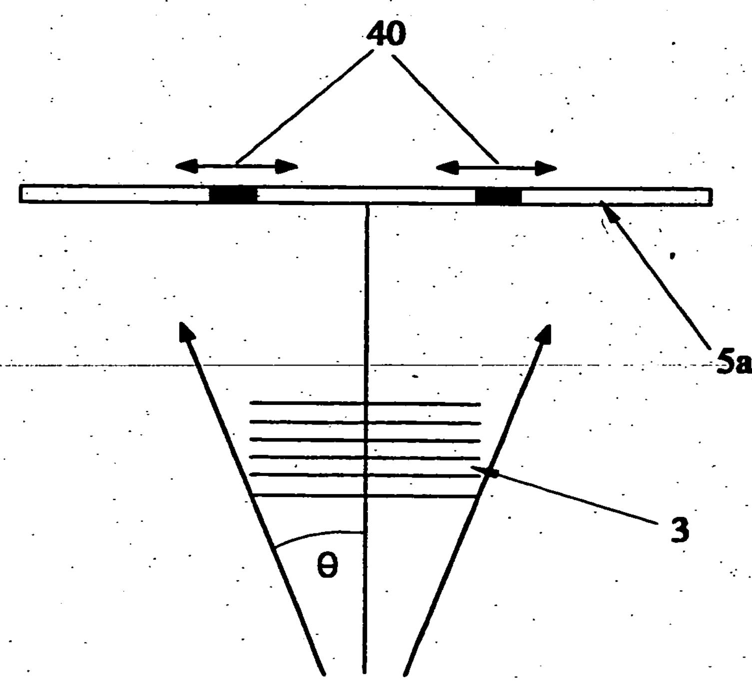

FIG. 4

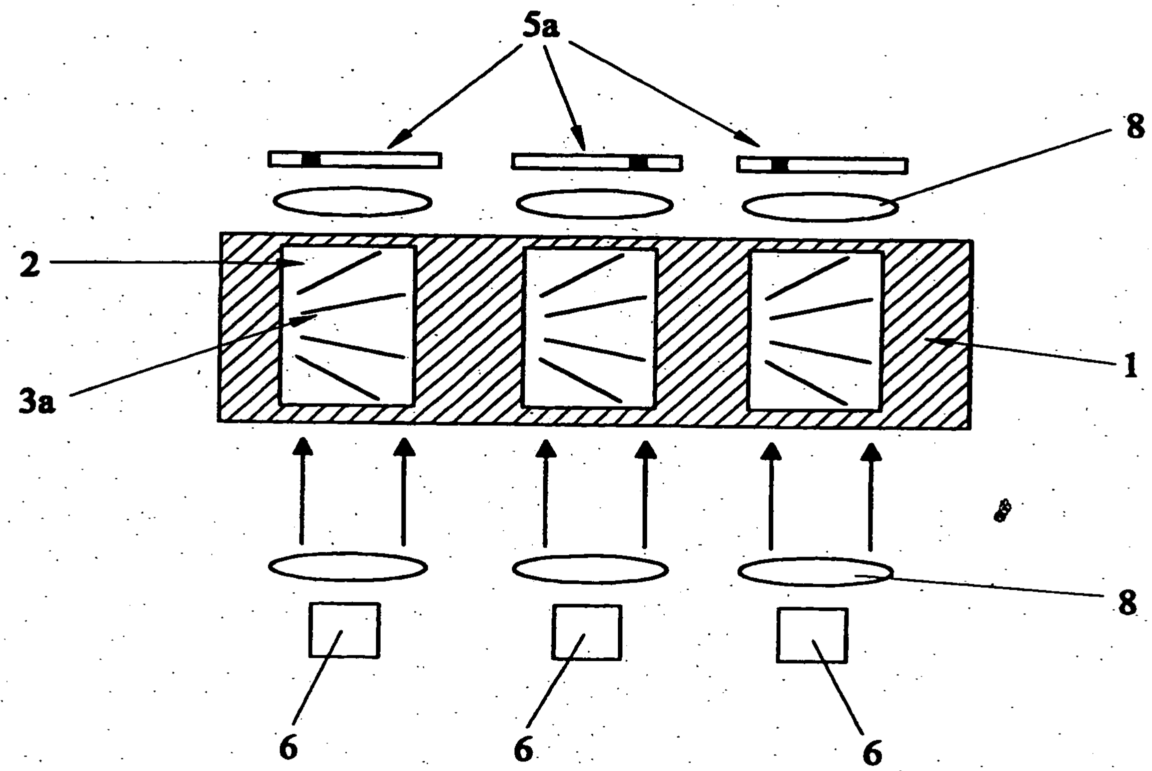

FIG. 5

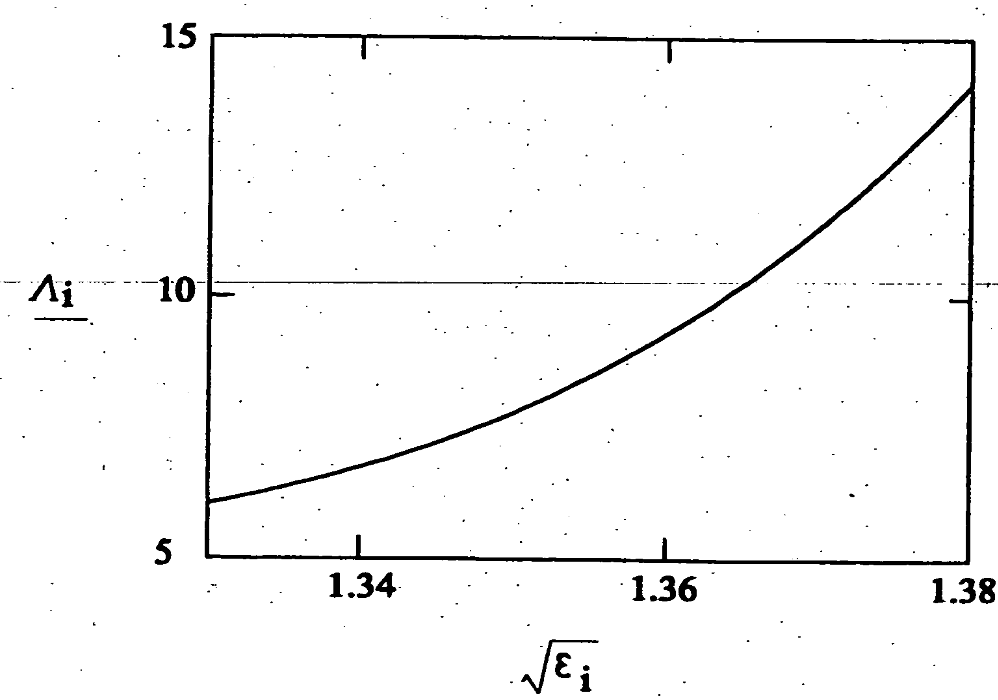

FIG. 6